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Impact of cryoconcentration on casein micelle size distribution, micelles inter-distance, and flow behavior of skim milk during refrigerated storage

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ABSTRACT

Cryoconcentration combined with a cascade effect was used to concentrate skim milk up to 25.12% total dry matter. Size, shape, and inter-micellar distance of casein micelles were characterized by ZetasizerNano-ZS, transmission electron microscopy, and ImageJ analyses. Flow properties of the cryoconcentrated skim milk were evaluated during 5 weeks of storage under refrigerated condition at 4 °C. Milk color was also evaluated according to the L^*, a^*, and b^* system. The cryoconcentrated skim milk obtained after three cryoconcentration cycles was characterized by a monomodal distribution of its micelles with a tendency to smaller casein micelles. Approximately 60% of the total micellar volume was occupied by the casein micelles with a size of 100–200 nm, less than 18% of the volume with a size of 50–100 nm and only less than 1% was occupied by micelles with a size >350 nm. This result shows that cryoconcentration changed the distribution of the mean size of the casein micelles to smaller units. No significant difference was observed on the inter-micellar distance. Cryoconcentration significantly improved the color of skim milk by increasing the L^* value up to 67 which was similar to that of whole milk. Transition from a Newtonian to a non-Newtonian behavior was observed from the fourth week storage with a slight increase of casein micelle size.

Industrial relevance: A concentration procedure of skim milk based on a complete block cryoconcentration technique was proposed. Application of this sub-zero technology permitted the concentration of skim milk total dry matter up to 25%. The casein micelle size was positively affected by moving the major part of the micelles toward the smaller size, whereas the inter-micellar distance was not affected. This new knowledge can be exploited in milk-based products to enhance the product stability. The cryoconcentrated skim milk color was positively affected since its L^* value, which represents the milk whiteness, was significantly improved. The flow behavior of the cryoconcentrated milk was of Newtonian type up to 4 weeks of storage at 4 °C. The generated knowledge in this study can be easily used by the milk processing industry in order to make stable milk product with high dry matter content without adding milk powder, which negatively affects the product sensory properties (floury consistency).

1. Introduction

The use of concentration processes in dairy industry can significantly contribute to enhance the overall efficiency of milk processing since huge quantities of milk can be reduced by concentrating the total dry matter of some specific components such as proteins, yielding advantages in terms of processing, packaging, transportation, and handling Keshani, Luqman Chuah, Nourouzi, Russly, & Jamilah, 2010. The selection of a convenient concentration process depends on the required level of concentration, the impact of the process on products quality, available energy resources, and the relative cost of the process. Sometimes, a combination of different concentration processes is also used (Morison & Hartel, 2006). Currently, there are several concentration methods available for enhancing milk concentration such as vacuum evaporation, reverse osmosis, ultrafiltration, and cryoconcentration in its different variants (freeze concentration) Miyawaki, Liu, Shirai, Sakashita, & Kagitani, 2005. Cryoconcentration of skim milk is a process of concentrating the solid matter contained in a the aqueous phase by removing part of water in a form of ice Aider, de Halleux, & Melnikova, 2009. The ice formation can be achieved by different ways such as suspension crystallization, progressive freeze concentration, and complete block cryoconcentration Gunathilake, Dozen, Shimmura, & Miyawaki, 2014; Iritani, Katagiri, Okada, Cao, & Kawasaki, 2013. Among these techniques, the complete block cryoconcentration is the

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simplest one for milk concentration and it is based on a controlled freezing followed by a controlled passive or assisted thawing.

This technology, being operated at sub-zero temperature conditions, is attractive for heat sensitive liquid foods since it allows retaining the nutritional quality and aromatic compounds within the product. This particularity is attributed to the low operating temperatures, which are suitable to avoid the degradation of the sensitive liquid food components such as heat labile proteins, vitamins, and volatiles (Flesland, 1995; Ghizzoni, Del Popolo, & Porretta, 1995, and the absence of a liquid–vapor interface. Actually, it is regarded as highly promising separation process of water from liquid foods without affecting the quality and properties of other components (Fellows, 2000). Considering these advantages, cryoconcentration technology has been investigated by several researchers for its application to a variety of liquid foods, such as milk, milk whey, fruit juices, maple sap, and saline solutions Di Cesare, Cortesi, & Martini, 1993. Cryoconcentrated milk is a dairy product that may be utilized as an intermediate material for sterilized, sweetened condensed milk, and Greek-type yoghurt production or as a final product for the consumer or use in different food formulations.

However, even if cryoconcentration technology seems to offer several advantages in comparison with other concentration techniques such as heat evaporation and membrane concentration (Thijssen, 1970; Thijssen & Van Der Malen, 1981), a high dehydration of casein micelles by water removal processes is of particular importance (De Kruif, 1999; Morris, Foster, & Harding, 2000; Walstra, 1979), since it can increase the volume fraction of dispersed particles and the inter-micelles interactions Bienvenue, Jimenez-Flores, & Singh, 2003. The latter (inter-micelles interactions) is very important since the casein micelles have the greatest impact on the milk macroscopic and functional properties Liu, Dunstan, & Martin, 2012, and they are the main contributors to the viscosity of milk (Walstra & Jenness, 1984) and significantly influence the cheese yield. Thus, any factor that alters the aggregation state of casein micelle, such as pH, concentration, and salt balance, undoubtedly affect the viscosity of milk (Bienvenue et al., 2003). In skim milk, the continuous phase viscosity is largely determined by lactose concentration, whereas the volume fraction of suspended material is determined by proteins such as casein micelles, dissociated caseins, native whey proteins, and denatured whey proteins (Anema, 2008; Jeurnink & De Kruif, 1993). It has been reported that when cryoconcentration is used, the increase of the total dry matter in the concentrated phase is accompanied by an increase of the amount of lactose entrapped in the ice crystals (Aider & Ounis, 2012). However, little is currently known how the physico-chemical properties of the casein micelles change in response to cryoconcentration.

A better understanding of the physico-chemical properties of cryoconcentrated milk and the changes occurring with progressively increasing cryoconcentration level is needed. This is necessary to further understand the dynamics of structure changes during cryoconcentration and ultimately better determine the main principles ruling the processing of cryoconcentrated milk under different storage conditions. Many studies have been conducted on the viscosity of concentrated milk prepared by heat evaporation (Vélez-Ruiz & Barbosa-Cánovas, 1998), ultrafiltration Karlsson, Ipsen, Schrader, & Ardö, 2005, or powder reconstitution Alexander, Rojas-Ochoa, Leser, & Schurtenberger, 2002; Anema, 2008; Dahbi, Alexander, Trappe, Dhont, & Schurtenberger, 2010, and some flow properties of freeze-concentrated skim milk were reported (Chang & Hartel, 1997). By contrast, limited information is available on the effects of freezing procedures such as the cryoconcentration on the micelle size, the inter-micellar distance (spacing between casein micelles), and the product flow properties during storage.

Hence, the purpose of this study is (1) to evaluate how cryoconcentration combined with a cascade affect influences the size of casein micelles, the inter-micelle distance, as well as the cryoconcentrated skim milk color, and (2) to establish the impact of the cryoconcentration procedure on the rheological properties of cryoconcentrated skim milk during refrigerated storage. The evaluation of the influence of temperature and concentration on the apparent viscosity was also performed during 5 weeks of storage at 4 °C.

2. Materials and methods

2.1. Skim milk and cryoconcentration procedure

Pasteurized skim milk was purchased from Natrel (Agropur Cooperative, Quebec, Canada) and was used as the start material. Skim milk proximate composition was the following: total dry matter, 9.24 ± 0.15%; lactose, 4.91 ± 0.21%; total protein, 3.54 ± 0.17%; and ash content, 0.79 ± 0.11%. The initial pH value was 6.5 ± 0.15.

The cryoconcentration procedure was carried out by applying the cascade principle (effect) as reported by Aider and Ounis (2012). To do this, glass bottles of 9.3 cm inner diameter and 15 cm height were used. The bottles were filled with 1000 ml skim milk and were frozen in a freezer at −20 ± 2 °C. After 24 h of freezing, the thawing step was carried out under simple gravitational separation of the concentrate from the ice block at ambient temperature (23 ± 1 °C). The collected fraction was maintained at near-zero temperature by immersing the collection bottle in ice water. This procedure avoided any risk of bacterial growth during the thawing period. At this step, 500 mL of the initial frozen volume was thawed and collected (50% of the initial volume). This fraction constituted the concentrated phase of the *1st cryoconcentration cycle*. The same procedure was repeated with the collected concentrated solution, which was used as a feed solution for the *2nd cryoconcentration cycle*. At the end of the second cryoconcentration cycle, 50% of the thawed solution was collected. This procedure was repeated at the *3rd cryoconcentration cycles*. Each concentrate at a given cycle was used as feed solution for the next cycle. To avoid any bacterial growth during the storage of the collected samples, sodium azide (0.01% w/w) was added to the skim milk as a preservative.

2.2. Analyses

2.2.1. Total dry matter

Total dry matter for all samples was determined by measuring weight loss upon drying in an oven at 100 °C under vacuum until constant weight (24 h) and expressed as dry matter content/total weight in %. The accuracy of the measurements was verified by a freeze drying control of some samples. No noticeable difference was find between the measurements.

2.2.2. Total proteins

Total protein content of each sample was determined by Dumas combustion method by using an FP-528 Leco apparatus (Leco Corporation, St. Joseph, MI, USA). The instrument was calibrated with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. The percentage of total protein content was calculated from nitrogen content by multiplying by a factor 6.38 (IDF, 2002).

2.2.3. Ash and mineral fraction analysis

Ash content of different skim milk samples and ice fractions was measured by the incineration method in a muffle furnace at 550 °C for 20 h. The specific mineral analysis (Ca, P, Mg, Na, and K) was carried out by the inductively coupled plasma method (ICP, Optima 4300 DV, Perkin-Elmer, Norwalk, CT, USA) with the following wavelengths: 317.933, 396.847, and 393.366 nm for Ca; 285.213, 280.271, and 279.553 nm for Mg; 766.490 nm for K; and 589.592 and 588.995 nm for Na (Carnovale, Britten, Couillard, & Bazinet, 2015).

2.2.4. Concentration factor and process efficiency

The concentration factor for each component (proteins, ash, specific minerals) at each cryoconcentration cycle was calculated as a ratio of its